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BACKGROUND AEROSOLS AND BW DETECTION: PROBLEMS AND SUGGESTED SOLUTIONS

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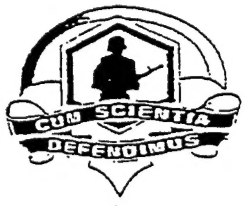

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Detection of Biological Warfare (BW) Agents is a formidable task, exacerbated by the fact that some microbes can be highly infective. Thus, only a few microbes delivered to an appropriate site can cause disease. Because of this issue, detectors need to have very high sensitivity. To further complicate things, the atmosphere frequently contains abundant biological and nonbiological particles that can interfere with the detection process, thus creating false alarms. In this report, the authors briefly review the difficulties that background aerosols cause in the detection process and suggest possible ways of alleviating these difficulties.

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PREFACE

The work described in this report was performed under Project No. 206023. This work was started in March 2002 and completed in June 2002.

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BACKGROUND AEROSOLS AND BW DETECTION: PROBLEMS AND SUGGESTED SOLUTIONS

1. INTRODUCTION

The background atmosphere contains biological and nonbiological material that can interfere with detecting Biological Warfare (BW) agents. These Bioaerosols are comprised of bacteria, viruses, spores, pollen, fungi, insect parts, etc.[1,2,3] These rich diversifications of microbes also contain all the microbe breakdown products that are in the process of further decay. In addition, not all the microbes are viable, and many of them available for collection are not named, and thus are unknowns. This situation is made more complicated by their continual variation in concentration in time and space, a phenomena that is not well understood. Some of the factors affecting the background include: time of day, season, local meteorology, human activities, land use, geographic location, and a host of other, as yet, unknown factors.

2. BW DETECTION AND THE BACKGROUND - PROBLEM DEFINITION

Figure 1 illustrates the problem the background presents for BW detection. Close to the BW dissemination point, the number concentration of the BW agent particles* (red dots in Figure 1) is larger than the number concentration of the background aerosol particles (green dots in Figure 1). Hence, detection is not a problem. As the BW cloud travels down wind and disperses, the background concentration remains the same (more or less), while the number concentration of the agent particles diminishes. The same problem is also illustrated in Figure 2. As noted in Figure 2, usually the desired sensitivity for BW detection falls within the background concentration. To put this another way, when the BW agent can be seen by the eye and the means of dispersal observed, identifying and detecting it as a biological agent is easier to determine. As the material disperses into the atmosphere and floats its way down wind, the concentration diminishes. In many situations, as soon as the material mixes with the indigenous microbes, it becomes less and less concentrated. Recall, for a moment, that only a few microbes may cause infection, and this condition forms the nature of the problem faced by detector systems - low concentration of highly infective microbes that are greatly outnumbered by the native microbes. Separating these different microbes from each other, or more directly separating the target microbes from the background microbes for detection and identification is the real nature of the task presented.

Figure 3 shows examples of biological aerosols that can be found in the background atmosphere. These aerosols can range in size from a fraction of micrometer to several tens of microns. This figure represents several issues regarding the difficulty of separating target microbes from the rest of the mix. First, some of these are found all over the world. Some species of fungi have vegetative parts that when broken apart are similar in size to their spores, both sexual and asexual to make it more interesting. Some of the bacteria have

*Note that BW agents are aerosol particles, in contrast to CW agents that can be either gas (vapor), liquid, or solid aerosols, depending on the agent, on the ambient temperature, and on the dissemination method.

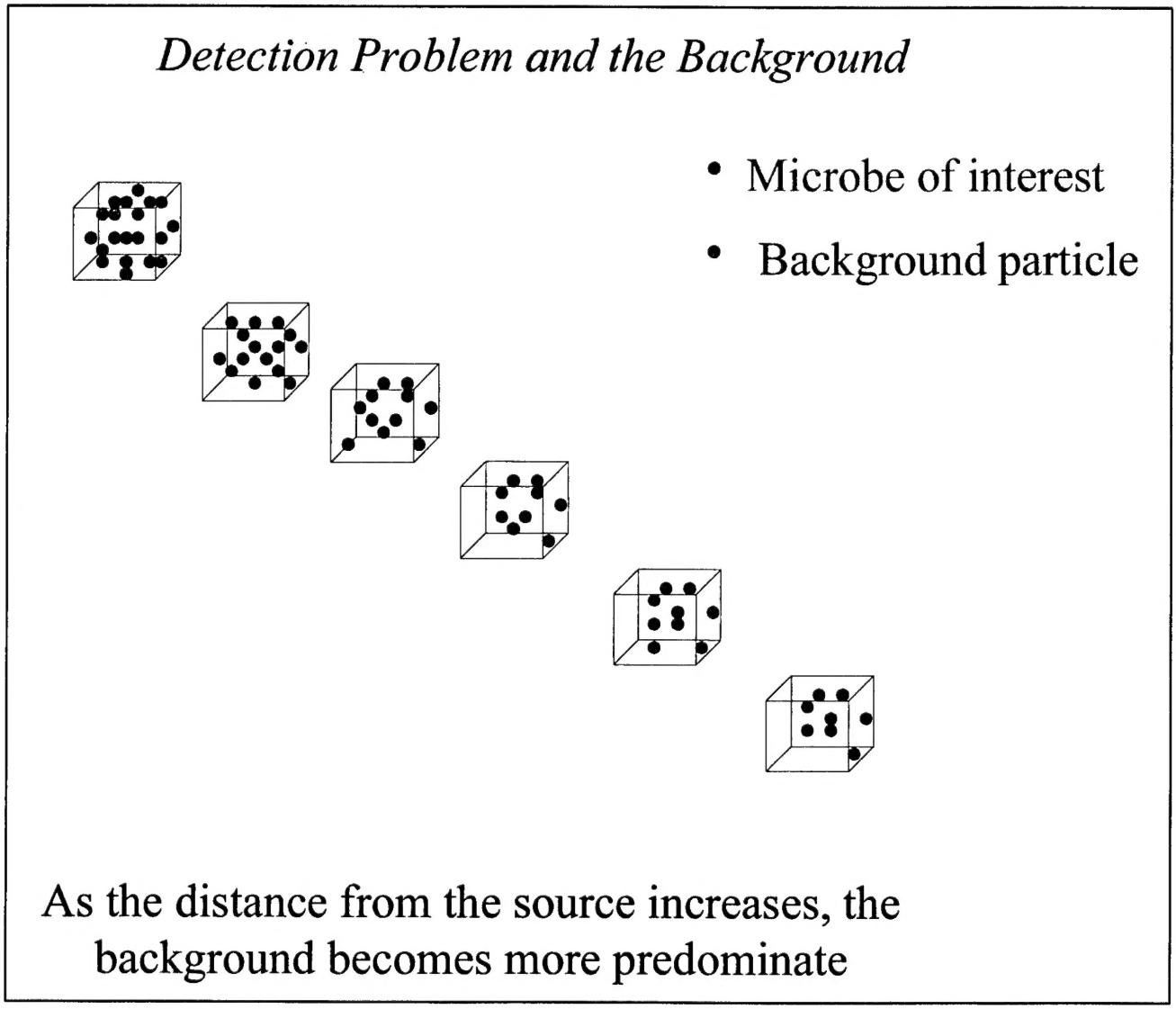

Figure 1. Pictorial Illustration of the Background and the BW Detection Problem

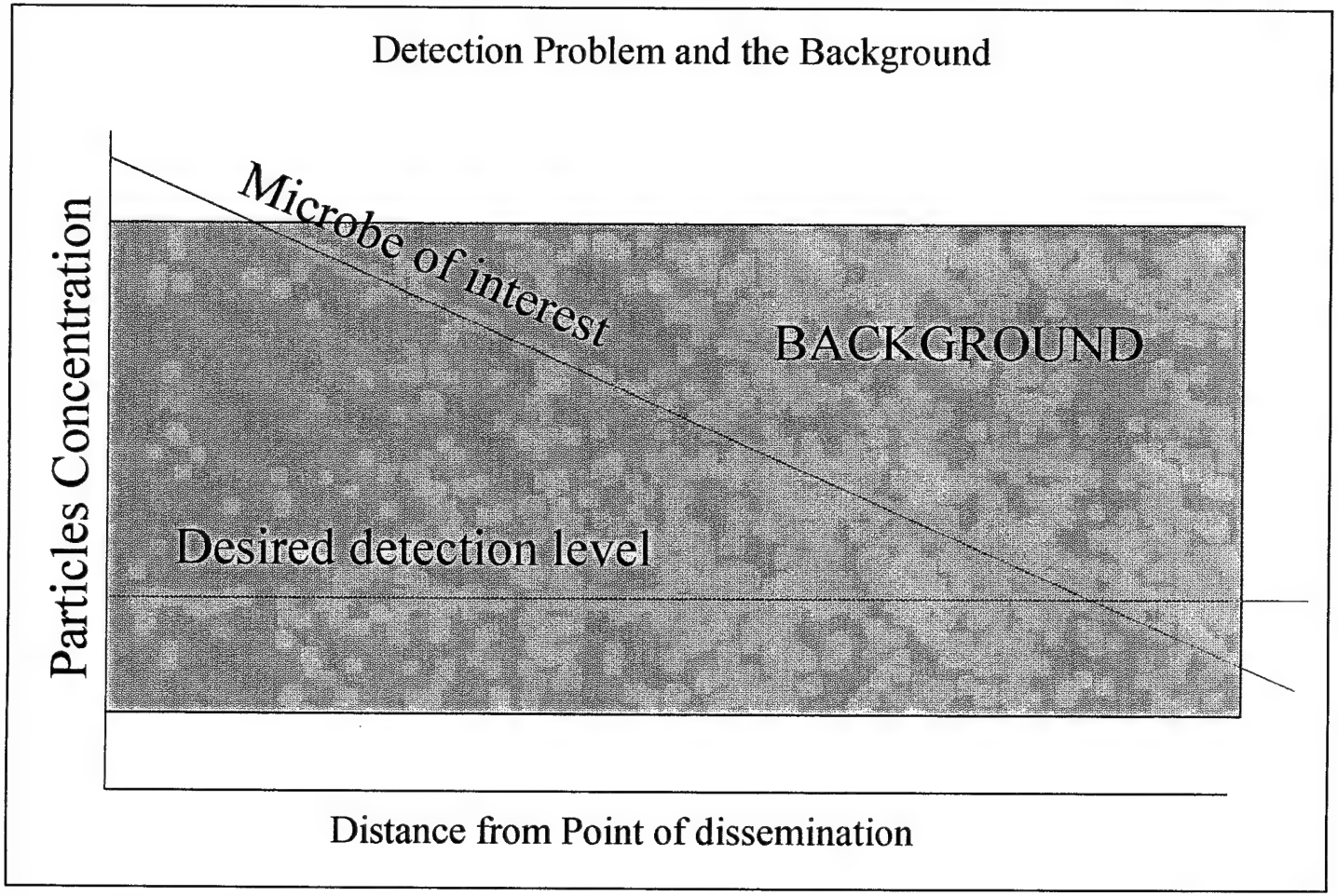

Figure 2. Pictorial Illustration Comparing Minimum Detection Level with Common Background

Figure 3. Example of Bioaerosols Found in Nature.
(Picture provided by courtesy of Dr. Charles Wick, ECBC)

projections that can also dry and break apart, forming smaller particles. Combined with enormous numbers of these microbes, the detection and separation challenges begin to take shape.

Various factors affect the background concentration of nonbiological and biological aerosols. Biological aerosols exhibit diurnal and seasonal cycles. The concentration of both depends on geography and land use (desert, urban, rural, forest, agricultural activity, etc.), human activities, etc. Some of the variability is not well understood, and only small fractions of the biological species have been identified. This does not imply that the issue of sampling, detecting, and identifying requires that all competing microbes be identified and cataloged. Not at all. What is implied is a need to develop and test our collectors and detectors under natural real world conditions.

3. DISCUSSION

Sources of biological aerosol are both natural and man-made. Soil and plants are a major source of bioaerosols.[4,5,6] Human activities contribute greatly to the atmospheric aerosol and bioaerosol load (e.g., vehicular traffic results in resuspension of particles, and water treatment plants are a good source of bioaerosols of various types).

Figures 4-8 show several examples of background information collected by different means and at different times and locations. Figure 4 shows an example of how the background particle number concentrations can vary within very short periods. As can be seen, the number concentration of the particles can more than double within a few minutes. Figure 5 illustrates how the particle concentration can vary spatially within short distances of several hundreds of meters.* †

Figure 6 shows the number concentration of cultureable bacteria particles. Again, the number concentration of particles containing cultureable bacteria can vary by a factor of 2 or more over a relatively short time-span. It must be emphasized that bacterial particles comprise only a small fraction of the total bioaerosols. In addition, only a small fraction of the total bacteria can be cultured.

Figures 7 and 8 show the response of two developmental BW detection systems in the outdoor background. Figure 7 shows the response of a Pyrolysis Gas Chromatograph/Ion Mobility Spectrometer (Py/GC-IMS) that is under development at ECBC.[7] In this detector, particles are collected and deposited onto a quartz wire over several minutes (nominally 3-5 min). Following the collection, the material collected on the wire is pyrolized by passing current through the wire and raising its temperature to 350 °C within 5 sec. As a result of the Pyrolysis, biological material produces various semivolatile compounds. The mixture of vapors is separated to its components by passing through a chromatographic column into a time of flight ion mobility spectrometer after passing through an ionizing volume. Some classes of bioaerosols give characteristic signatures. For example, gram positive spores produce Picolinic acid that is a decomposition product of dipicolonic acid.[8] Work is now in progress to determine whether other classes of bioaerosols also have typical signatures.

Figure 8 shows an example of data collected outdoors by the Single Particle Fluorescence Analyzer (SPFA), a developmental detector at the Naval Research Laboratory (NRL), Washington, DC.[9,10,11] The SPFA counts and sizes all entering particles using a cw laser for optical scattering. In addition, it is designed to differentiate particles of biological origin (also known as bioaerosols) from all other aerosols. It achieves this by using a second pulsed laser at a 266 nm wavelength to excite UV fluorescence from each biological particle as they pass by. Because certain amino acids (principally tryptophan and tyrosine) fluoresce when excited near their absorption peaks in the UV region of the spectrum, a strong particle fluorescence signal indicates the presence of biological material, since these amino acids occur in nearly all proteins. Therefore, this spectroscopic characteristic was used to indicate the presence of aerosols of biological origin.[12,13,14]

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*Birenzvige, A., Research and Technology Directorate, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, 1999, unpublished data.

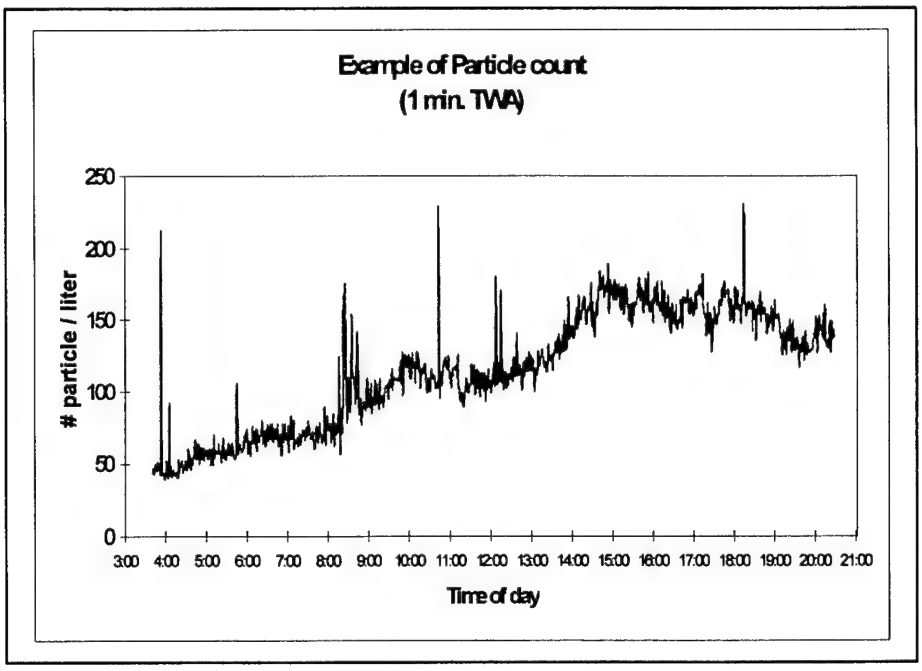

Figure 4. Example of Particle Counts (Number Concentration) During the Day

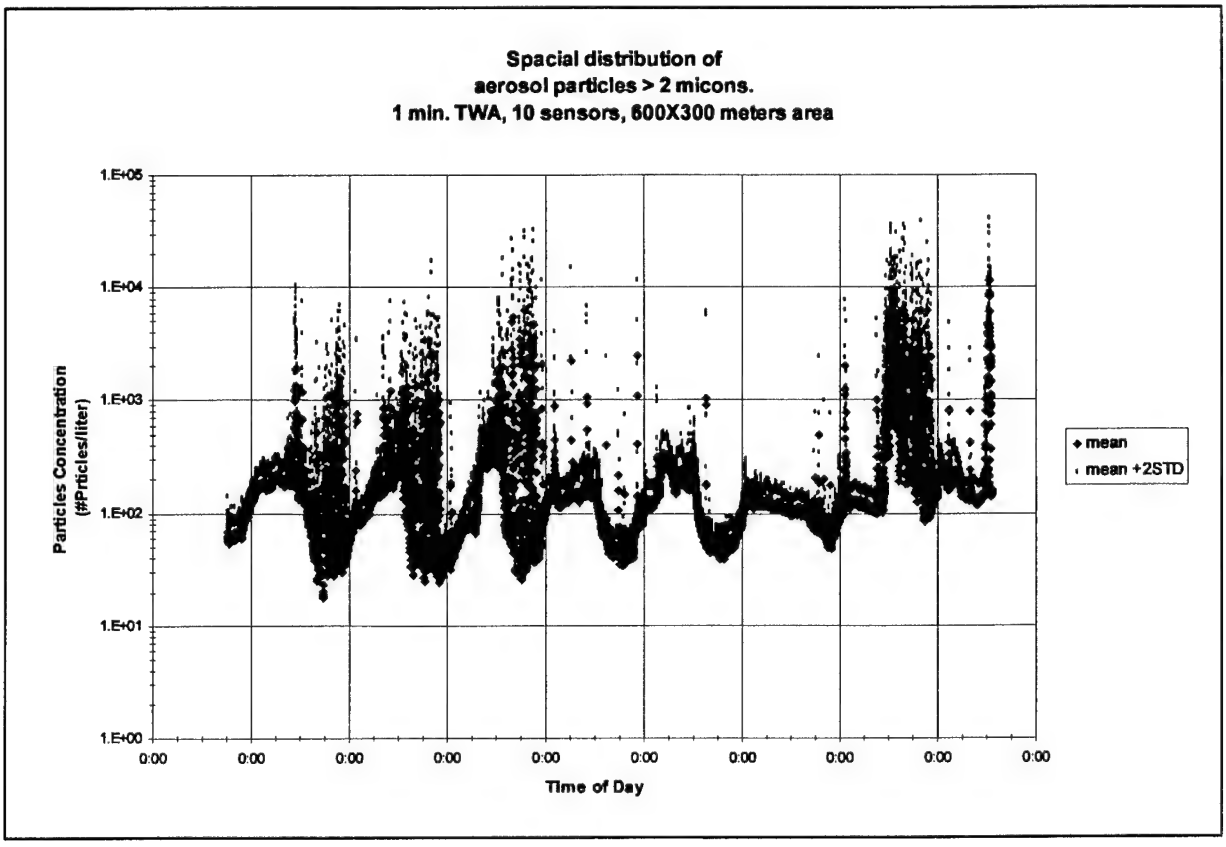

Figure 5. Example of the Spatial Distribution of Environmental Aerosols

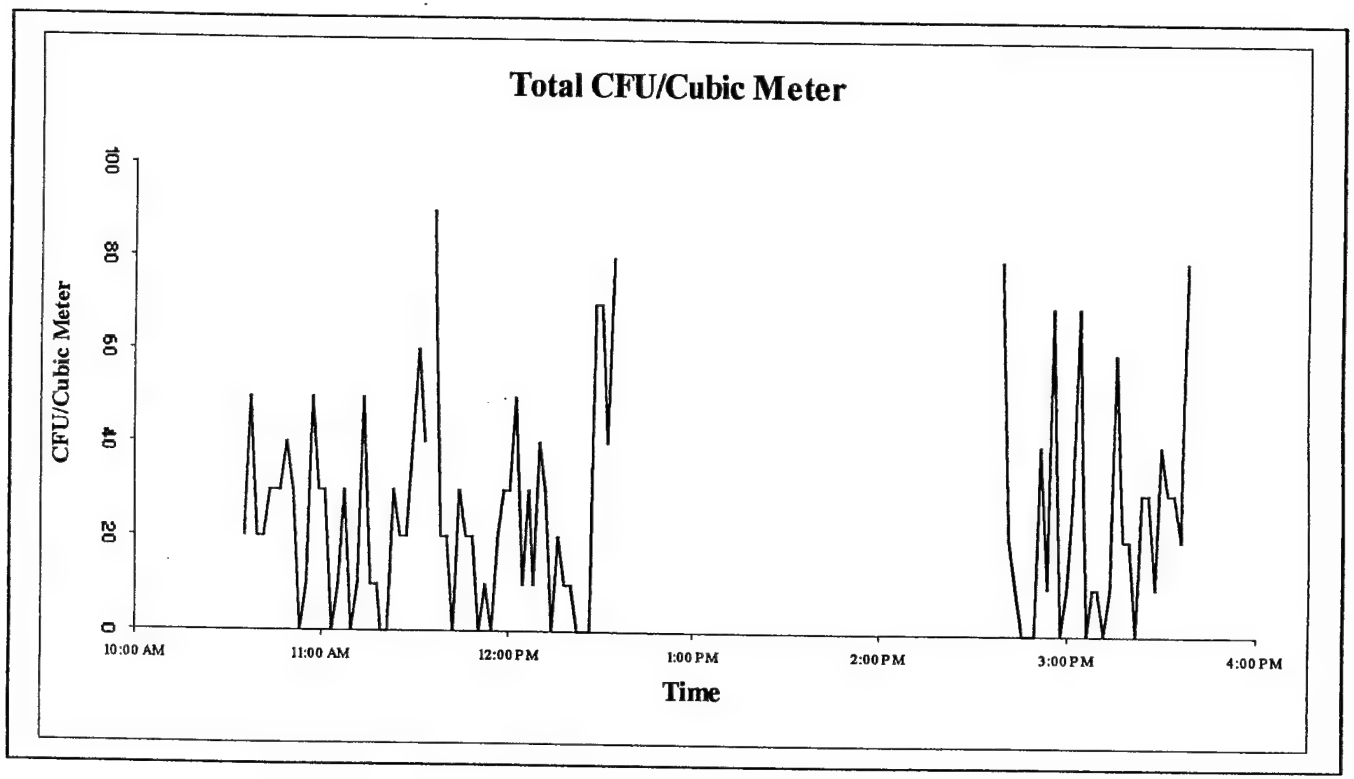

Figure 6. Example of Cultureable Bacteria Concentration in the Air.
(Note that only 1%-10% of the total bacteria in the respirable air can be cultured.)

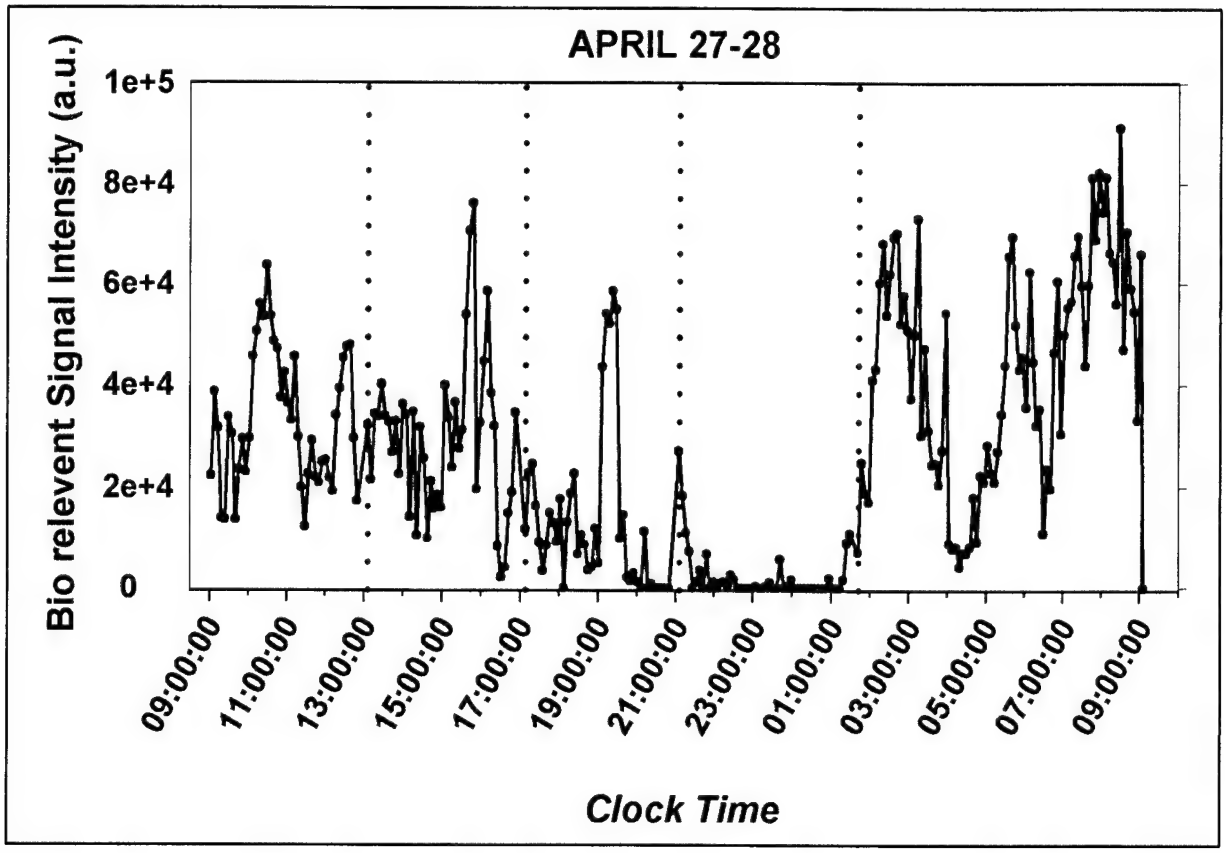

Figure 7. Example of a Py-GC/IMS (Developmental BW Detector at ECBC) Signature in the Background
(Graph provided by courtesy of Dr. Peter Snyder, ECBC)

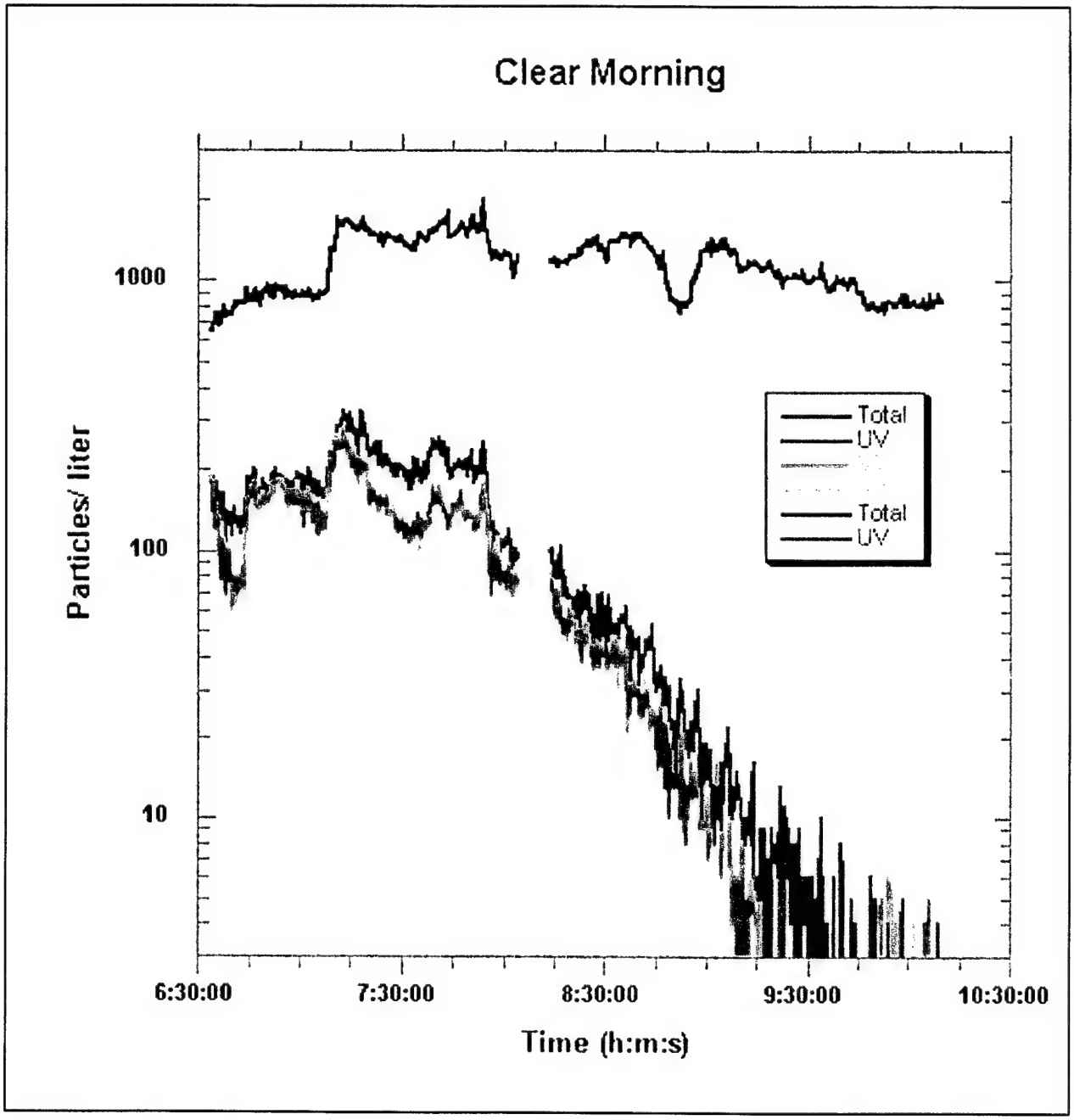

Figure 8. Example of the SPFA Signature in the Background
(Graph provided by courtesy of D. Jay Everso, NRL).

4. CONCLUSIONS AND RECOMMENDATIONS

Because of their high virulence, the detection requirements for BW agents are challenging. In general, most people cite requirements of 10 Agent Containing Particles per Liter of Air (ACPLA). As obvious from Figures 4, 6, and 8, ten ACPLA is within the natural variability of background bioaerosols.

Biological Warfare (BW) detectors are developed and tested for their ability to detect BW agents in the clean laboratory. Field tests are conducted during the Joint Field Trials (JFT). Usually the JFT are conducted at Dugway Proving Ground (DPG), DPG, UT, which is a high desert pristine area with a very low indigenous bioaerosols' background.

Normally, when BW agent detectors operate in a less pristine area rich in bioaerosols, they generate a large number of false alarms. False alarms are the nimbus of the system, and too many render such a system impotent. The main factor for reducing false alarms is the high cost in unit readiness when the alarm is given. Typically, troops at risk assume a higher Mission Oriented Protective Posture (MOPP) status, a situation that reduces their ability to fight. How long troops can remain in MOPP is further influenced by the temperature and humidity. Field commanders take a dim view of a high false alarm rate because of these considerations and the impact on unit mission. Hence, it is very important to limit the false alarm rate to a minimum.* Frequently, the material developer increases the "threshold" value for sounding the alarm. This has the effect of increasing the probability that a low-level attack will go undetected. In other words, it increases the rate of false negatives, which to a field commander is even worse.

The primary investigator used a number of respirable aerosol counters ($2-10\mu m$ diameter) to monitor the spatial distribution of these aerosols. He was able to show that when the counters were placed far enough apart from each other (300 - 500 m) such that their data were not correlated, and using the ensemble of counters as a unified system, he could detect a low level simulated attack by fusing the individual counters data[15,16,17] with very low rates of false positives and false negatives. Common sense and statistical theory indicate that similar results could be obtained by using several collocated detectors that use orthogonal technologies. The principal question that needs to be resolved is: which combination of technologies could provide the best solution?

To answer this question, the authors of this report recommend that BW sensors be evaluated in the real world environment. The authors also recommend that a "background test center" be established in a location that has an active bioaerosol background (e.g., a suburban area that is surrounded or in the vicinity of fields and woods). Sensors should be brought to this background test center for evaluation early in the development stage (even at the "bread board" stage). The evaluation should be performed for extended periods (preferably for a period of at least 1 year). The rate of false positives could be established for the particular technology singly and in combination with other technologies. To evaluate the probability of detection, the

*Since 9-11, there has been increased concern of bioterrorist attacks. Limiting the false alarms to absolute minimum is even more important in the civilian arena than it is during military operations.

investigators recommend using modeling and simulation. Detector response to a given threat can be simulated and superimposed onto a real background using various discriminating algorithms.

We believe this approach will enable management to down select the best suite of detectors. Furthermore, it will provide a tool to evaluate cost benefit ratios for evaluating new technologies early in the development cycle.

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